Estimating Losses of Dry Matter from Simulated Rainfall on Bermudagrass and Orchardgrass Forages Using Cell Wall Components as Markers

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ABSTRACT

Previous methodologies to measure losses of dry matter (DM) in wilting hays subjected to natural or simulated rainfall have relied generally on gravimetric techniques, resulting in variable and questionable estimates of DM loss. The objective of this study was to evaluate the use of fiber components and acid detergent insoluble ash (ADIA) as internal plant markers for accurately predicting losses of DM in bermudagrass [*Cynodon dactylon* (L.) Pers.] and orchardgrass (*Dactylis glomerata* L.) forages that were damaged by simulated rainfall. For both forages, concentrations of neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose (HEMI), cellulose (CELL), lignin, and ADIA generally increased with the amount of simulated rainfall in primarily linear patterns. Recoveries of all internal markers were high ($\geq$952 g kg^{-1}) and were not affected by simulated rainfall for either forage ($P \geq 0.06$). Predicted losses of DM increased in primarily linear patterns with simulated rainfall for both forages when concentrations of NDF, ADF, HEMI, CELL, and ADIA were used as internal markers. Linear regressions of predicted losses of DM on values determined gravimetrically were good ($r^2 \geq 0.73$; $P \leq 0.03$) when concentrations of any fiber constituent or ADIA were used to calculate losses of DM; however, NDF was an especially effective internal marker ($Y = 1.12X - 5$; $r^2 = 0.97$; $P < 0.01$).

Hay producers in the USA often must make management decisions that attempt to minimize losses of forage DM during the wilting period that follows cutting and precedes baling. Prevailing weather conditions throughout many areas of the USA include high relative humidity and/or a high probability of rainfall when hay production is feasible. The time interval associated with field curing of hay is often prolonged by high relative humidity (Moser, 1995), which subsequently increases the probability of damage to the hay crop before baling. The impacts of rain damage on the nutritive value of hay crops have been outlined in several research reports (Collins, 1982; Rotz and Abrams, 1988; Smith and Brown, 1994). Generally, soluble cell components are leached from plant tissues (Sundberg and Thylén, 1994), and the primary leachates are nonstructural carbohydrates (Collins, 1982), which account directly for losses of DM from the hay crop and indirectly for increases in concentrations of insoluble cell wall components (Collins, 1982, 1983; Rotz et al., 1991).

Methodologies used to estimate losses of DM in experiments with rain-damaged forages generally have been based on gravimetric techniques, but these techniques have been problematic. In several reports (Rotz et al., 1991; Rotz and Abrams, 1988), mowed forages were weighed into wire-mesh trays before natural rainfall occurred or before artificial rainfall was applied via various simulation techniques. When using these methods, Rotz et al. (1991) and Rotz and Abrams (1988) noted numerous problems, including negative estimates of DM loss from wilting alfalfa (*Medicago sativa* L.). Similarly, Gordon et al. (1969) reported highly variable, and sometimes negative, estimates of DM loss for rain-damaged alfalfa and orchardgrass forages. Negative estimates of DM loss falsely suggest that DM was created due to rainfall damage. Clearly, the gravimetric techniques used to determine losses of DM in these experiments have produced questionable results. Rotz et al. (1991) have suggested that these errors were associated with small fluctuations in estimates of the initial DM concentration of each experimental forage. Another source of error may be the incorrect assumption that all forage in the basket, tray, or windrow is uniform and that this DM concentration adequately represents the forage before application of simulated or natural rainfall.

Furthermore, all DM loss cannot be related specifically to leaching of soluble forage components during rainfall events. Summaries of past work compiled by Rotz and Muck (1994) suggest that respiration within plant cells approaches nil only when forages are dehydrated to between 260 and 400 g kg^{-1} of moisture. Therefore, plant respiratory processes may continue between pre- and postrainfall sampling as well as during oven drying at relatively low temperatures (<60°C) that do not prohibit the subsequent analysis of forage fiber components (Van Soest, 1982). These types of errors may inflate, rather than deflate, estimates of DM loss. Regardless of the source of error, alternative methodologies for determining DM loss are needed to ensure that accurate estimates are obtained.

One technique that has been used by a limited number of scientists to estimate losses of DM in rain-damaged forages is based on the principle that most cell wall components are insoluble in water (Van Soest, 1982). Although concentrations of cell wall constituents generally increase in response to rain damage, these changes are associated indirectly with decreased concentrations of

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Published in Agron. J. 96:1680–1687 (2004).

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Abbreviations: ADF, acid detergent fiber; ADIA, acid detergent insoluble ash; CELL, cellulose; DM, dry matter; HEMI, hemicellulose; NDF, neutral detergent fiber.

cell-soluble constituents (particularly sugars) that are leached from the forage (Collins, 1982); in theory, the actual amount or pool of cell wall components should remain unchanged. Therefore, insoluble cell wall components should be potentially useful as internal markers to accurately predict losses of DM. Based on this premise, Fonnesbeck et al. (1986) suggested that losses of DM in rain-damaged forages could be determined by the equation:

$$\text{DM loss (g kg}^{-1}) = [1 - (CW_I/CW_R)] \times 1000 \text{ g kg}^{-1}$$

where CW_I = cell wall concentration before the rainfall event and CW_R = cell wall concentration after the rainfall event. Using this equation, Fonnesbeck et al. (1986) reported calculated DM losses of 46 and 97 g kg^{-1} for alfalfa hay that incurred 5 and 20 mm, respectively, of natural rainfall.

Alternatively, ADIA is commonly used as an internal marker to estimate fractional rates of digesta passage (Waldo et al., 1972), and this fraction also may be useful as an internal marker for measuring DM losses from wilting forages. Similarly, Salo and Virtanen (1983) calculated losses of DM ranging from 120 to 290 g kg^{-1} in rain-damaged cool-season grass hays using concentrations of lignin as an internal marker. While methodologies of these types may prove superior to gravimetric techniques, there is very little information available that describes and/or verifies the legitimacy of their use. The objective of this study was to evaluate the efficacy of using insoluble cell wall constituents and ADIA as internal markers to predict losses of DM in bermudagrass and orchardgrass forages damaged by simulated rainfall.

EXPERIMENTAL PROCEDURES

Rainfall Simulation

Two separate studies were conducted. One study utilized a second cutting of 'Benchmark' orchardgrass harvested on 20 June 2001 at the University of Arkansas Forage Research Area in Fayetteville (36°05′ N, 94°10′ W; elevation of 394.5 m). The second study utilized common bermudagrass harvested as hay from an adjacent field at the same research site during the summer of 2001. Hays were packaged in small rectangular bales and stored in an open-air pole barn until January 2002. On 4 January 2002, samples were taken from duplicate bales of each forage and chopped to a 2.5-cm length using a standard 61- by 61-cm paper cutter. Chopped, 8-g samples of each forage were weighed into 24 dacron bags (10 by 20 cm, 53-μm pore size; ANKOM Technol., Fairport, NY), sealed with an impulse heat sealer (Model CD-200; Natl. Instrument Co., Baltimore, MD), and dried to a constant weight in a forced-air oven at 55°C. Bags were removed from the drier and immediately weighed (hot) before the forage particles could absorb water from the atmosphere. This procedure was used to obtain the most accurate estimate possible of the total amount of forage DM in each bag, without compromising subsequent analyses of fiber components by drying at temperatures >60°C (Van Soest, 1982).

Twenty bags containing bermudagrass forage were placed under a custom-built rainfall simulator and separated into four blocks. Each block designation was associated with a single corresponding sprinkler head on the rainfall simulator, and artificial rainfall was applied to bags at a constant rate of 838 mm h^{-1}. One bag from each block was removed from under the simulator in specific time intervals that resulted in applications of 51, 102, 203, 406, or 610 mm of artificial rainfall. An undamaged control consisted of four bags of bermudagrass hay that did not receive applications of simulated rainfall (0 mm). Although the rate of application was extremely high compared with typical rates of natural rainfall, much of the applied water was shed by the dacron bags; however, all forages receiving simulated rainfall, regardless of rainfall increment, were completely saturated when they were removed from under the simulator. It should be noted that dacron bags of this type are designed specifically for evaluating ruminal disappearance kinetics of ground forages; therefore, they are permeable to water, and theoretically, there should be no loss of insoluble forage particles as a result of applying simulated rainfall.

After bags were wetted by simulated rainfall, they were allowed to drip dry for 0.5 h and then dried to a constant weight under forced air (55°C). Bags were removed from the oven and immediately weighed (hot) to determine the final amount of forage DM contained within each individual bag. By sealing the test forages within dacron bags, the amount of forage DM in each bag could be determined without the need to subsample or quantitatively transfer forages into paper bags or other containers for drying, thereby risking additional experimental error. Actual losses of DM were calculated as differences in initial and final amounts of DM within each bag and are reported as a proportion of the initial amount of DM. At the conclusion of the bermudagrass study, the exact same procedures were used to apply simulated rainfall to dacron bags filled with orchardgrass forage.

In using these procedures, our goals were twofold. One goal was to generate different amounts of DM loss that covered the range expected under normal field conditions as a result of rain damage. Although the test forages used in this study were extremely dry when simulated rainfall was applied, DM losses generated by these techniques (Table 1) generally covered the range

Table 1. Actual losses of dry matter in bermudagrass and orchardgrass forages determined by gravimetric techniques after application of graduated amounts of simulated rainfall.

Simulated rainfall	Bermudagrass	Orchardgrass
mm	g kg^{-1}	
0	0	0
51	10	33
102	13	54
204	27	59
406	30	85
610	47	98
SEM†	3.2	6.0
Response	$P > F$	
Linear	<0.01	<0.01
Quadratic	0.16	<0.01
Cubic	0.02	0.02
Quartic	0.44	0.03

† SEM, standard error of the mean.

expected for wilting hay crops damaged by natural rainfall (Gordon et al., 1969; Rotz and Abrams, 1988). Second, proper validation of any marker requires a standard technique or other precise and accurate measurements for comparison. Based on the problematic and erratic estimates of DM loss described previously for forages placed in wire baskets and subjected to simulated rainfall (Rotz et al., 1991; Rotz and Abrams, 1988), it was clear that additional precautions were necessary to determine DM losses accurately so that marker systems could be verified. Specifically, these precautions included (i) sealing forages in dacron bags and drying under forced air before wetting to determine the initial amount of DM in each bag; (ii) using the same procedure to determine the amount of DM in each bag after wetting, which eliminated any need for subsampling or transfer steps; and (iii) utilizing dacron bags designed to assess ruminal disappearance kinetics of ground forages to ensure that there was no loss of insoluble forage particles during any of the experimental procedures.

It should be emphasized clearly that these precautions were used only for the purpose of marker verification. After markers are verified clearly, they should be useful for measuring DM loss in controlled studies similar to those described previously that utilize wire baskets or trays and some type of simulated rainfall. However, the effectiveness of these marker systems in controlled studies will still depend on the complete recovery of any shattered leaf material following the application of simulated rainfall. Clearly, this should be more problematic with legumes than with grasses.

Chemical Analysis of Forage

All dry forage samples were ground through a Wiley mill (Arthur H. Thomas, Philadelphia, PA) to pass a 1-mm screen and analyzed in duplicate for concentrations of NDF, ADF, HEMI, CELL, and lignin. The NDF, ADF, HEMI, CELL, and lignin analyses were conducted sequentially, using the batch procedures outlined by ANKOM Technology Corporation (Fairport, NY). Sodium sulfite and heat-stable α-amylase were not included in the NDF solution. Hemicellulose was determined from the difference in residual weights following sequential incubation of each forage in neutral and acid detergent. Similarly, CELL was determined from the difference in residual weights following further extraction of ADF residues for 3 h in 72% (w/w) sulfuric acid. After extraction in sulfuric acid was completed, residues were ashed at 500°C for 8 h in a muffle furnace. The portion of residue lost on ignition was defined as acid detergent lignin, which was subsequently reported as a proportion of the original sample weight. A second set of samples was analyzed in duplicate for concentrations of ADF using nonsequential procedures. These ADF residues were ashed in a muffle furnace at 500°C for 8 h, and the weight of residual ash was used to calculate ADIA.

Recoveries of Potential Markers

The utility of any marker depends on its ability to remain unaffected by the application of treatment, and complete recovery of the marker is expected following treatment. Each of the markers in this study was evaluated by calculating the amount of each marker on a weight basis (g), both before and after simulated rainfall treatments. Recoveries for each potential marker were calculated as:

$$\text{Marker recovery (g kg}^{-1}) = [\text{marker recovered (g)}/\text{marker before treatment (g)}] \times 1000 \text{ g kg}^{-1}$$

To get the best possible estimate of the initial concentration of each potential marker in the experimental forages, subsamples of each chopped forage were taken regularly (12 per forage) as the dacron bags were filled. These subsamples were composited, thoroughly mixed, ground, and analyzed for fiber components and ADIA as described previously. Marker recoveries from dacron bags receiving no simulated rainfall sometimes differed slightly from complete recovery (1000 g kg^{-1}), and these differences reflect sampling, handling, and laboratory errors during the experimental procedures. These small errors also are reflected in DM losses predicted by markers for dacron bags receiving no simulated rainfall.

Calculated Dry Matter Loss

Concentrations of fiber components before and after rainfall treatments were used to estimate losses of DM using the equation suggested by Fonnesbeck et al. (1986), which was described previously.

Statistical Analysis

Data for the bermudagrass and orchardgrass trials were analyzed independently as a randomized complete block design with four replications based on positioning under the rainfall simulator. The effects of simulated rainfall on actual DM loss, concentrations and recoveries of all potential markers, and predicted DM losses were evaluated by trend analysis using the GLM procedures of SAS (SAS Inst., 1989). The sums of squares were partitioned into linear, quadratic, cubic, and quartic effects and tested for significance with the residual error mean square.

Agreement between the marker-based estimates of DM loss and actual DM losses determined by gravimetric procedures was tested by linear regression. A slope of unity and an intercept of zero would indicate ideal agreement between methods. Initially, tests of homogeneity (PROC GLM) were conducted to detect differences in parameter estimates (intercept and slope) between bermudagrass and orchardgrass forages. If both the slope and intercept did not differ ($P > 0.05$) across forages, data were combined, and a common regression equation was reported. If the regression lines for each forage were not homogenous ($P < 0.05$), a separate regression equation was generated for each forage. The REG procedure of SAS (SAS Inst., 1989) was used to establish each regression equation. An additional test statement was included to evaluate whether slope = 1. Throughout the study, statistical significance was declared at $P \leq 0.05$.

Table 2. Concentrations of fiber components and acid detergent insoluble ash (ADIA) in bermudagrass forage as affected by graded levels of simulated rainfall. Fiber components were determined sequentially.

Simulated rainfall	NDF†	ADF‡	HEMI§	CELL¶	Lignin	ADIA
mm			$g\ kg^{-1}$			
0	737	308	423	270	41.6	17.6
51	742	317	424	278	41.9	18.1
102	748	321	426	279	43.9	18.4
204	750	323	427	281	44.6	18.9
406	751	323	428	281	44.0	19.2
610	772	325	444	283	44.0	19.8
SEM#	3.7	3.9	5.0	3.3	1.99	0.71
Response			$P > F$			
Linear	<0.01	0.01	0.01	0.03	0.38	0.02
Quadratic	0.32	0.11	0.30	0.20	0.40	0.62
Cubic	0.03	0.13	0.38	0.18	0.60	0.61
Quartic	0.86	0.49	0.83	0.44	0.83	0.96

† NDF, neutral detergent fiber.
‡ ADF, acid detergent fiber.
§ HEMI, hemicellulose.
¶ CELL, cellulose.
# SEM, standard error of the mean.

RESULTS AND DISCUSSION

Actual Dry Matter Losses

The cubic ($P = 0.02$) and linear ($P < 0.01$) terms described the actual losses of DM from dacron bags containing bermudagrass forage damaged by simulated rainfall (Table 1). For orchardgrass (Table 1), actual DM losses increased with simulated rainfall, and all polynomial terms were significant ($P \leq 0.03$). Actual DM losses ranged from 0 to 98 g kg^{-1}, which would be expected under field conditions (Gordon et al., 1969; Rotz and Abrams, 1988); however, the primary use of gravimetric determinations of DM loss in this experiment was to provide a valid reference method for evaluating predicted losses of DM calculated with concentrations of fiber components and ADIA as internal markers.

Concentrations of Markers

The concentration of NDF was 35 g kg^{-1} greater in bermudagrass forage receiving 610 mm of simulated rainfall compared with the 0-mm treatment, and this response was explained with significant cubic ($P = 0.03$) and linear ($P < 0.01$) terms (Table 2). Concentrations of ADF, HEMI, and CELL increased linearly ($P \leq 0.03$) by 17, 21, and 13 g kg^{-1}, respectively, with simulated rainfall, but other polynomial terms were not significant ($P \geq 0.11$). Lignin was not affected by treatment ($P \geq 0.38$) although numerical increases were observed as simulated rainfall increased. Acid detergent insoluble ash increased linearly ($P = 0.02$) with simulated rainfall, but the overall range of estimates was small (17.6 to 19.8 g kg^{-1}).

Concentrations of NDF in orchardgrass forage increased in response to simulated rainfall; all polynomial terms were significant ($P \leq 0.05$; Table 3). The concentration of NDF increased by 73 g kg^{-1} in forage that received the most rainfall (610 mm) relative to the 0-mm control. Concentrations of ADF and CELL increased by 32 and 30 g kg^{-1}, respectively, and exhibited a quadratic increase ($P \leq 0.02$) with simulated rainfall. In both cases, the linear term also was significant ($P < 0.01$). Concentrations of HEMI, lignin, and ADIA increased by 40, 8.8, and 1.6 g kg^{-1}, respectively, in a linear relationship ($P \leq 0.01$) with simulated rainfall, but all other polynomial effects were not significant ($P \geq 0.12$).

Increased concentrations of fiber components clearly

Table 3. Concentrations of fiber components and acid detergent insoluble ash (ADIA) in orchardgrass forage as affected by graded levels of simulated rainfall. Fiber components were determined sequentially.

Simulated rainfall	NDF†	ADF‡	HEMI§	CELL¶	Lignin	ADIA
mm			$g\ kg^{-1}$			
0	640	326	315	288	38.8	19.3
51	670	335	332	301	40.1	20.1
102	678	346	332	305	40.4	20.0
204	686	346	340	311	42.6	19.7
406	704	357	347	317	44.4	20.1
610	713	358	355	318	47.6	20.9
SEM#	5.1	3.7	5.1	4.1	2.24	0.30
Response			$P > F$			
Linear	<0.01	<0.01	<0.01	<0.01	<0.01	0.01
Quadratic	<0.01	0.01	0.12	0.02	0.91	0.45
Cubic	0.04	0.31	0.21	0.23	0.80	0.21
Quartic	0.05	0.20	0.42	0.42	0.88	0.15

† NDF, neutral detergent fiber.
‡ ADF, acid detergent fiber.
§ HEMI, hemicellulose.
¶ CELL, cellulose.
# SEM, standard error of the mean.

Table 4. Recoveries of potential internal markers [fiber components and acid detergent insoluble ash (ADIA)] in bermudagrass forage after application of graded levels of simulated rainfall. Fiber analysis was by sequential methodology.

Simulated rainfall	NDF†	ADF‡	HEMI§	CELL¶	Lignin	ADIA
mm			g kg^{-1}			
0	1000	1000	1000	1000	1000	999
51	996	1020	991	1019	997	1016
102	1001	1030	994	1021	1040	1034
204	990	1020	983	1012	1042	1043
406	988	1018	982	1010	1026	1058
610	998	1008	999	999	1008	1071
SEM#	4.5	12.1	11.8	11.7	46.9	40.1
Response			$P > F$			
Linear	0.48	0.79	0.92	0.40	0.94	0.17
Quadratic	0.06	0.18	0.18	0.34	0.46	0.67
Cubic	0.46	0.34	0.86	0.47	0.74	0.78
Quartic	0.69	0.34	0.98	0.30	0.88	0.92

† NDF, neutral detergent fiber.
‡ ADF, acid detergent fiber.
§ HEMI, hemicellulose.
¶ CELL, cellulose.
# SEM, standard error of the mean.

were expected for bermudagrass and orchardgrass forages that were damaged by simulated rainfall, and the range of increases agrees generally with previous work. Collins (1991) found that concentrations of NDF and ADF increased by 75 and 50 g kg^{-1} DM, respectively, in alfalfa hay that was soaked for 0.5 h in deionized water. Similarly, Collins (1982) reported increased concentrations of NDF, ADF, and lignin for alfalfa, red clover (*Trifolium pratense* L.), and birdsfoot trefoil (*Lotus corniculatus* L.) in response to natural and artificial rainfall damage. Respective concentrations of NDF increased by 61, 108, and 98 g kg^{-1} for these three forages after 2.5 cm of water was applied after a 24-h wilting period and again after 48 h of wilting. In addition, Fonnesbeck et al. (1986) reported increases of 42 g kg^{-1} for concentrations of cell walls in alfalfa hay in response to 20 mm of artificial rainfall; in addition, concentrations of CELL, HEMI, and lignin increased by 32, 5, and 10 g kg^{-1}, respectively. Previous work by Collins (1982) clearly shows that increased concentrations of fiber components occur concomitant with reduced concentrations of total sugars and nonstructural carbohydrates, which are leached from the forage. Therefore, increased concentrations of NDF and other fiber components occur via an indirect mechanism, rather than as the result of additional deposition of plant fiber.

Estimated Recoveries of Internal Markers

Simulated rainfall did not affect ($P \geq 0.06$) the recovery of any internal marker that was evaluated for either forage (Tables 4 and 5). In theory, proper validation procedures for internal markers require the estimation of marker recovery from experimental materials after treatments are applied. These types of marker systems have been used extensively in conventional digestion trials to estimate digestibility coefficients and passage kinetics for feedstuffs consumed by ruminant animals (Sunvold and Cochran, 1991; Ellis et al., 1994). In theory, internal markers rely on the assumption that the marker is not affected by experimental treatments and that it is completely recovered following treatment. Therefore, when expressed as a proportion of initial quantities, marker recoveries should be approximately 1000 g kg^{-1}. In this study, recoveries of all internal markers were high ($\geq$952 g kg^{-1}) at all levels of simulated rainfall, suggesting that these components may be acceptable for use as internal indicators of DM loss (Tables 4 and

Table 5. Recoveries of potential internal markers [fiber components and acid detergent insoluble ash (ADIA)] in orchardgrass forage after application of graded levels of simulated rainfall. Fiber analysis was by sequential methodology.

Simulated rainfall	NDF†	ADF‡	HEMI§	CELL¶	Lignin	ADIA
mm			g kg^{-1}			
0	1000	1015	1002	1003	1001	999
51	1011	1010	1021	1014	998	1004
102	1003	1021	1000	1004	986	978
204	1009	1016	1018	1018	1035	962
406	1007	1020	1011	1009	1049	952
610	1005	1008	1018	999	1101	976
SEM#	7.1	14.0	12.6	11.1	59.0	16.6
Response			$P > F$			
Linear	0.91	0.81	0.57	0.64	0.12	0.11
Quadratic	0.56	0.58	0.91	0.35	0.82	0.06
Cubic	0.81	0.84	0.70	0.86	0.99	0.80
Quartic	0.77	0.96	0.96	0.83	0.70	0.65

† NDF, neutral detergent fiber.
‡ ADF, acid detergent fiber.
§ HEMI, hemicellulose.
¶ CELL, cellulose.
# SEM, standard error of the mean.

Table 6. Losses of dry matter in bermudagrass forage predicted on the basis of concentrations of fiber components or acid detergent insoluble ash (ADIA) after application of graded levels of simulated rainfall.

Simulated rainfall	NDF†	ADF‡	HEMI§	CELL¶	Lignin	ADIA
mm			$g\ kg^{-1}$			
0	0	−1	−1	−1	−14	−13
51	6	29	1	29	−12	19
102	14	42	8	34	51	41
204	17	46	10	38	64	66
406	18	47	12	39	48	83
610	46	54	46	46	54	110
SEM#	4.8	12.0	11.5	12.0	50.1	41.3
Response			$P > F$			
Linear	<0.01	0.01	0.01	0.03	0.33	0.03
Quadratic	0.37	0.10	0.35	0.19	0.39	0.51
Cubic	0.03	0.11	0.37	0.17	0.55	0.61
Quartic	0.87	0.46	0.85	0.42	0.80	0.96

† NDF, neutral detergent fiber.
‡ ADF, acid detergent fiber.
§ HEMI, hemicellulose.
¶ CELL, cellulose.
# SEM, standard error of the mean.

5). In some cases, recoveries exceeded 1000 g kg^{-1}, and this was especially true for lignin, for which recoveries reached a maximum of 1101 g kg^{-1} in orchardgrass forage. Similarly, recoveries of ADIA reached a maximum of 1071 g kg^{-1} for bermudagrass forage, but this problem was not observed generally for orchardgrass.

Predicted Losses of Dry Matter

For bermudagrass forage (Table 6), predicted losses of DM estimated with NDF as an internal marker increased from 0 to 46 g kg^{-1} in a cubic ($P = 0.03$) relationship with simulated rainfall; the linear term also was significant ($P < 0.01$). Losses of DM predicted using concentrations of ADF, HEMI, and CELL increased linearly ($P \leq 0.03$) in response to simulated rain damage, but losses of DM predicted with lignin increased only numerically ($P = 0.33$). With ADIA, predicted losses of DM increased linearly ($P = 0.03$); however, the range of estimates using ADIA (−13 to 110 g kg^{-1}) was approximately twice as large as the ranges observed for the other markers.

For orchardgrass (Table 7), predicted losses of DM calculated with NDF as an internal marker increased from 0 to 102 g kg^{-1} over the entire range of simulated rainfall, and all polynomial terms were significant ($P \leq 0.04$). Losses of DM estimated with ADF and CELL increased in a quadratic ($P = 0.01$) pattern with a significant linear term ($P < 0.01$). Predicted losses of DM estimated with HEMI and lignin increased in linear patterns ($P \leq 0.01$) with simulated rainfall. When 610 mm of water was applied, predicted losses of DM determined with all fiber components (except lignin) were maximized between 91 and 112 g kg^{-1}; however, predicted losses were much higher (184 g kg^{-1}) when lignin served as the internal marker. In contrast, maximum losses (75 g kg^{-1}) based on ADIA were numerically lower than observed for the other potential markers.

In our study, both lignin and ADIA comprised a much smaller proportion of the total forage DM than did NDF, ADF, HEMI, and CELL, and procedures for quantifying lignin and ADIA are far more tedious and problematic than those for other fiber components. Predicting DM loss on the basis of relatively subtle differences in these concentrations may be problematic relative to NDF, which is easy to quantify and comprises a large proportion of the total forage DM. Previously,

Table 7. Losses of dry matter in orchardgrass forage predicted on the basis of concentrations of fiber components or acid detergent insoluble ash (ADIA) after application of graded levels of simulated rainfall.

Simulated rainfall	NDF†	ADF‡	HEMI§	CELL¶	Lignin	ADIA
mm			$g\ kg^{-1}$			
0	0	0	−1	0	−10	−2
51	44	28	51	43	30	35
102	56	58	52	54	26	32
204	67	59	73	72	69	21
406	91	89	93	90	124	38
610	102	91	112	94	184	75
SEM#	7.1	10.1	14.0	12.4	56.3	15.3
Response			$P > F$			
Linear	<0.01	<0.01	<0.01	<0.01	0.01	0.01
Quadratic	<0.01	0.01	0.09	0.01	0.91	0.57
Cubic	0.03	0.26	0.18	0.19	0.92	0.22
Quartic	0.04	0.17	0.33	0.36	0.94	0.18

† NDF, neutral detergent fiber.
‡ ADF, acid detergent fiber.
§ HEMI, hemicellulose.
¶ CELL, cellulose.
# SEM, standard error of the mean.

Salo and Virtanen (1983) reported much higher ranges (120 to 290 g kg^{-1}) of DM loss in wilting cool-season grass than those observed in our study when lignin was used to predict losses. These authors suggested that contamination of hay samples with residual organic matter and the potential decomposition of lignin might have led to inaccurate estimates of DM loss. An additional consideration in choosing an internal marker is that some fiber components, such as NDF and ADF, are likely to be included in nearly all evaluations of forage nutritive value; therefore, predicting DM loss via one of these internal markers would not require additional analytical costs.

Predicted versus Actual Dry Matter Losses

Statistics for predicted DM loss calculated on the basis of internal markers regressed on actual DM losses determined by gravimetric techniques are presented in Table 8. For both forage types, relationships between predicted and actual losses of DM were good ($r^2 \geq 0.73$; $P \leq 0.03$), regardless of which internal marker was used. Relationships were particularly good when concentrations of NDF were used to predict losses of DM ($Y = 1.12X - 5$; $r^2 = 0.97$; $P < 0.01$). In this relationship, the slopes and intercepts for the two forages did not differ ($P \geq 0.23$; data not shown), and data for the two forages were combined into a common regression equation.

For ADF and HEMI, linear regression lines for the two forage types were not homogenous due to relatively fine differences (15 and 10 g kg^{-1}, respectively; $P \leq 0.04$) in intercept; therefore, regression statistics for each forage are reported separately (Table 8). For orchardgrass forage, predicted DM losses estimated from ADF and HEMI related very well ($r^2 \geq 0.96$; $P \leq 0.01$) to actual losses of DM, but relationships were not as strong for bermudagrass ($r^2 \geq 0.74$; $P \leq 0.03$). Within each forage type, the slope and intercept were not different from unity ($P \geq 0.44$) and zero ($P \geq 0.14$), respectively.

When either CELL or lignin was used as an internal marker, relationships between predicted and actual losses of DM were strong (Table 8); however, the r^2 statistic was lower for lignin ($r^2 = 0.80$) than for CELL ($r^2 = 0.94$). In both cases, regression lines for individual forages were homogenous ($P \geq 0.281$; data not shown), and data were combined into a single ($N = 12$) relationship. For CELL, the slope (0.92) did not differ from unity ($P = 0.30$), but the intercept (10 g kg^{-1}) was greater ($P = 0.02$) than zero. Conversely, the estimated slope (1.62) based on lignin as an internal marker differed ($P = 0.04$) from unity, but the intercept (−11 g kg^{-1}) did not differ from zero ($P = 0.42$). Predicted losses of DM calculated from ADIA were closely related to actual DM losses in bermudagrass ($r^2 = 0.96$; $P < 0.01$) and orchardgrass ($r^2 = 0.73$; $P = 0.03$) forages. However, the regression lines were not homogenous between the two forage types, primarily because of the large differences ($P < 0.01$; data not shown) in the slope. The slope for bermudagrass (2.60) was far greater than unity ($P < 0.01$), but this did not occur for orchardgrass ($P = 0.11$).

Regressions of predicted DM loss on actual DM loss indicate that fiber components generally produced relatively accurate estimates of DM loss when they were used as internal markers. This was especially true within a specific forage type. As discussed previously, a valid prediction method should yield a slope of unity and an intercept of zero when estimates are regressed against those determined by the standard method. Parameter estimates produced when fiber components were used as internal markers generally met these expectations although both lignin and ADIA were clearly less accept-

Table 8. Linear regressions of dry matter losses predicted with various internal markers on actual losses measured gravimetrically for bermudagrass and orchardgrass forages.

Marker†	Forage‡	N§	Slope	SE_{slope}¶	P_{slope}#	Intercept	SE_{int}††	P_{int}‡‡	r^2	$P_{regression}$§§
						g kg^{-1}				
NDF	B	–	–	–	–	–	–	–	–	–
	O	–	–	–	–	–	–	–	–	–
	COMB¶¶	12	1.12	0.062	0.08	−5	3.0	0.12	0.97	<0.01
ADF	B	6	1.02	0.305	0.96	15	8.0	0.14	0.74	0.03
	O	6	0.99	0.065	0.88	0	4.1	0.99	0.98	<0.01
	COMB	–	–	–	–	–	–	–	–	–
HEMI	B	6	0.93	0.218	0.75	−7	5.7	0.58	0.82	0.01
	O	6	1.09	0.106	0.44	3	6.7	0.64	0.96	<0.01
	COMB	–	–	–	–	–	–	–	–	–
CELL	B	–	–	–	–	–	–	–	–	–
	O	–	–	–	–	–	–	–	–	–
	COMB	12	0.92	0.077	0.30	10	3.7	0.02	0.94	<0.01
Lignin	B	–	–	–	–	–	–	–	–	–
	O	–	–	–	–	–	–	–	–	–
	COMB	12	1.62	0.259	0.04	−11	12.6	0.42	0.80	<0.01
ADIA	B	6	2.60	0.254	<0.01	−5	6.7	0.54	0.96	<0.01
	O	6	0.61	0.187	0.11	0	11.9	0.98	0.73	0.03
	COMB	–	–	–	–	–	–	–	–	–

† NDF, neutral detergent fiber; ADF, acid detergent fiber; HEMI, hemicellulose; CELL, cellulose; ADIA, acid detergent insoluble ash.
‡ B, bermudagrass; O, orchardgrass; COMB, regression includes data from both forages.
§ Number of treatment means in the linear regression.
¶ Standard error of the slope.
# Probability that the slope = 1.
†† Standard error of the intercept.
‡‡ Probability that the intercept = 0.
§§ $P > F$ for the overall regression model.
¶¶ Indicates regression lines for orchardgrass and bermudagrass forages were homogenous, and data for both forages were combined.

able. These techniques appear to provide unique opportunities to dramatically improve the reliability of DM loss estimates for forages that are damaged by simulated or natural rainfall. This may especially be true for grasses that are not necessarily prone to excessive leaf shatter during rainfall events; however, complete recovery of fragile, shattered leaves would be an essential requirement for using these internal markers in studies with legumes.

CONCLUSIONS

Concentrations of most fiber components increased in response to simulated rainfall and were completely recovered from bermudagrass and orchardgrass forages after simulated rainfall damage. In addition, relationships between predicted and actual losses of DM were good ($r^2 \geq 0.73$) when concentrations of any marker were used to calculate DM losses; NDF ($r^2 = 0.97$) was especially effective across both forages. Therefore, concentrations of cell wall components appear to be ideal for use as internal indicators of DM loss in rain-damaged forages. Lignin and ADIA were less acceptable as internal markers, probably because their concentrations are relatively low and quantification procedures are tedious and variable. Reliable estimates of DM loss in rain-damaged forages may best be predicted using concentrations of NDF due to the relatively rapid and inexpensive nature of that procedure and because it is generally determined for most experimental forages as part of routine forage-testing procedures.

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